FINAL PROGRESS REPORT

Project Title: Development of Diagnostic Reagents and Candidate Vaccines for Porcine Circovirus type 2

Objectives:

1. Create through transformation a cell line to grow PCV2 in high titer for antigen production

2. Clone and express the PCV2 coat protein gene in bacteria for recombinant antigen production

3. Develop an ELISA serology test to detect PCV2 specific antibodies in porcine serum samples

4. Develop Potential candidate vaccines for PCV2

Generation of a Stable Cell Line of Porcine Origin. Primary fetal porcine kidney cells were transfected with a plasmid, pSV3neo using calcium phosphate precipitation technique. PSV3neo was a pBR322-based plasmid containing the SV40 large T antigen gene, the neomycin resistance (neo^r) gene, and the SV40 origin of replication. The neo^r colonies were selected in the presence of G418 and were tested for expression of the SV40 large T-antigen by western blotting. A cell line, M12, was subsequently used for PCV2 replication.

Expression of PCV2 Coat Protein in Bacteria. The coat protein gene was amplified by polymerase chain reaction (PCR) using specific primers. The PCR product was inserted into a suitable bacterial expression and purification vector, pET30a (Invitrogen, Inc.) that drives the expression of the foreign insert fused to the histidine cassette by the T7 promoter. Unfortunately repeated attempts to express the full-length coat protein gene were unsuccessful. However, we were successful in expressing a 129R carbxy portion of the coat protein gene in large quantities. The truncated viral coat protein, as a fusion protein was purified by Ni^{++} affinity chromatography.

Development of ELISA to Detect PCV-Specific Antibody in Pig Serum Samples. 96-well microtiter plates were coated with various concentrations of either purified PCV or the bacterially expressed truncated coat protein and incubated with different dilutions of each serum sample to standardize ELISA for PCV2. A horseradish peroxidase (HRP)-conjugated goat anti-pig IgG (Bethyl Laboratories, Inc., Montgomery, TX) was used as the secondary antibody. ABTS (2,2' –azino-di-[3-ethylbenzthiazoline sulfonate) was used as a substrate and the optical density (OD) at 405 nm of each well will be measured with an ELISA reader The serum dilution showing an OD

reading of at least the mean OD+ 2 SD of negative control sera will be taken as the ELISA antibody titer. A number of known PCV2 positive and negative sera obtained from our previous swine inoculation studies as well as serum samples collected from various farms in Indiana were used to evaluate the sensitivity of this test. Recombinant coat protein was found excellent for monitoring PCV-specific antibody in pig serum samples by ELISA.

Potential Candidate Vaccines for PCV. PCV does not grow to high titers in cultured cells but we were successful in expressing the truncated-form of PCV coat protein gene in bacteria and this recombinant protein was easy to purify in large quantities. This recombinant protein could be tested as potential candidate vaccine for PCV using an appropriate adjuvant (e.g., muramyl dipeptide, alum or mineral oil) or a delivery vehicle (e.g., biodegradable alginate microparticles) as a vaccine formulation.